PATENT
Docket No.: **176/60901 (6-11402-968)**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Shohei Koide	Examiner: Shulamith H. Shafer
Serial No.	:	10/006,760	
Cnfrm. No.	:	2042	Art Unit: 1647
Filing Date	:	November 19, 2001	
For	:	METHOD OF IDENTIFYING POLYPEPTIDE MONOBODIES WHICH BIND TO TARGET PROTEINS AND USE THEREOF	

SECOND DECLARATION OF SHOHEI KOIDE UNDER 37 CFR § 1.132

I, SHOHEI KOIDE, declare as follows:

1. I am the inventor of the above-identified application.

2. I am a listed co-author of a presentation at the 5th Annual Scientific Symposium for the University of Rochester Cancer Center, Rochester, New York (October 3, 2000). A copy or the presentation abstract is attached hereto as Exhibit 1. My co-authors are Akiko Koide and Stacy Abbatiello.

3. The invention described and claimed in the above-identified application was conceived solely by me. In making the invention, experiments were conducted by me or under my direction.

4. Stacy Abbatiello, a lab technician in my laboratory at the time, was listed as a co-author on the presentation abstract due to her handling of routine laboratory work at my direction. Akiko Koide, a research associate in my laboratory, performed DNA construction and cell culture experiments according to my design and direction. Neither Stacy Abbatiello nor Akiko Koide contributed to the conception of the invention as described and claimed in the above-identified application.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 3/03/2009

Shohei Koide

Exhibit 1: Akiko Koide, "Selection of Monobodies that Recognize the Human Estrogen Receptor," University of Rochester Cancer Center 5th Annual Scientific Symposium, October 3, 2000, Abstract only.

ABSTRACT SUBMISSION FORM
UNIVERSITY OF ROCHESTER CANCER CENTER
5th ANNUAL SCIENTIFIC SYMPOSIUM
October 3, 2000
Memorial Art Gallery – Rochester, New York

SUBMISSION DEADLINE: August 7, 2000

Submission must be made electronically: e-mail: office@cpe.rochester.edu

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Indicate presentation format desired: X Poster Presentation __ Oral Presentation ____ Either

Was this research funded? Yes__ If yes, by what agency/agencies? NIH and US Army

Selection of Monobodies that Recognize the Human Estrogen Receptor

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We have developed a protein discovery system in which random peptides are displayed on the scaffold of a small beta-sheet protein, fibronectin type III domain (FN3). Loops connecting beta-strands of FN3 are randomized to generate a combinatorial library, and novel binding proteins can be selected from such a FN3 library using the phage-display technology. We term FN3-based binding proteins "monobodies."

Here we examined the potential of the yeast two-hybrid system for library screening. Compared to phage display, the yeast two-hybrid system is not commonly used for combinatorial library screening. However this *in vivo* technique might offer some advantages over *in vitro* selection methods such as phage display. These advantages may include stringent selection for specificity, and a possibility of selection against a target that is difficult to purify.

We chose the ligand (hormone)-binding domain (LBD) of the human estrogen receptor alpha (hER) as the target for selection. hER is a member of the nuclear hormone receptor superfamily, and it is a ligand-activated transcription factor. A majority of hER-positive breast tumors respond, at least initially, to antiestrogen therapies. Current anti-estrogen therapies are based on hormone antagonists, but the development of antiestrogen resistance is a serious concern in breast cancer treatment. Thus, hER inhibitors that function independent of the hormone-binding site may offer an alternative strategy in breast cancer treatment.

We prepared FN3 combinatorial libraries in the yeast two-hybrid system and performed library screening against hER LBD in the presence of an agonist or antagonist. We have obtained a number of monobodies that recognize the hER-estradiol (agonist) complex and/or the hER-4-OH tamoxifen (antagonist) complex. Monobodies that bind to hER could lead to therapeutic and/or diagnostic tools for breast cancer. Furthermore, this system could be used to screen estrogenic compounds such as environmental estrogens.

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